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Chromosomal analysis of two buffalo breeds of Mazani and Azeri from Iran

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Abstract

In the present study karyotype of Mazani river buffalo was studied in comparison with those of Azeri buffalo populations from Iran. Blood samples were taken from ten (5 males and 5 females) Mazani buffaloes and thirty (15 males and 15 females) Azeri buffaloes. The Mazani buffaloes belong to Mazendaran province and Azeri buffaloes belong to west and east Azerbaijan and Ardebil provinces. Blood lymphocytes cultured at 37ºC for 72 hours in the presence of phytohemagglutinin and the metaphase spreads were performed on microscopic slide. Giemsa was used to stain chromosomes. The Mazani and Azeri Buffalo exhibited the same karyotype with diploid number of 2n = 50. The fundamental numbers (NF) were 60 in male and female. The types of chromosome were 6 submetacentric, 4 metacentric and 40 telocentric which the X chromosome is the largest telocentric and the Y chromosome is one of the smallest telocentric chromosomes. The relative length of chromosomes ranged between 2.17% to 7.2% in Mazani buffalo, and also 2.21% to 6.55% in Azeri buffalo. No obvious abnormality was found among chromosomes. Therefore, based on the identified karyotype both Mazani and Azeri buffaloes are riverine.

Keywords: Karyotype, Chromosome, River buffalo, Idiogram

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Introduction

Buffalo (family *Bovidae* and tribe Bovini) can be divided into two main groups: *Bubalia* and *Syncerina*. *Bubalia* is also classified into Arni buffalo, Tamarao buffalo and Anona buffalo. Moreover, *Syncerina* consist of two subgroups called red buffalo and black buffalo. The arni buffalo is classified further into two groups, the river buffalo and the swamp buffalo according to its habitat (Miyake *et al*., 1980; Ahmad *et al*., 2004; Ali *et al*., 2012). The diploid chromosome number of the swamp buffalo is 48 (Harisah *et al*., 1989), and the diploid chromosome number of the river buffalo is 50 (Murali *et al*., 2009; Ali *et al*., 2012).

According to climate conditions, Iranian buffaloes consist of three main categories: 1) Azeri ecotype (Ardabil, Western and Eastern Azerbaijan provinces); 2) North ecotype (Guilan and Mazendaran provinces); and 3) Khoozestan ecotype (Khoozestan provinces) (Naserian and Saremi, 2007). Most of Iranian buffaloes live in the western and eastern Azerbaijan provinces and in Khoozestan province (Hasanzadeh and Monazzah, 2011). Buffaloes are bred due to their ability to producing milk and meat and draft power (Mirhoseini *et al*., 2005). Buffaloes have an important and essential role in the economy of rural families in Iran (Hasanzadeh and Orojee, 2003).It seemsthat Iranian buffaloes and Iraqi buffaloes have been originated from the same ancestor, due to phenotopic similarities. In addition Iranian river buffaloes in northwest of the country (West Azerbaijan), are phenotopically close to Mediterranean river buffaloes. So, it's considered that they may have descended from the common ancestors (Naserian andsaremi, 2007).

Cytogenetic study is a useful instrument in determining the standard karyotype of farm animals and helpful for recognition of chromosomal abnormalities (Ahmad *et al*., 2004). The animals with chromosomal abnormalities can be identified and removed from breeding stock (Ahmad *et al*., 2004).

The aim of this study was to determine the karyotype of two breeds of Iranian river buffalo and compare it with the karyotype of other river buffaloes from other countries.

Materials and methods

Sampling: Ten Mazani (5 males and 5 females) and thirty Azeri buffaloes (15 males and 15 females) were used for the chromosomal analysis. The Mazani buffalo samples were collected from of Mazendaran province located in the north of Iran and Azeri buffalo samples were collected from Ardabil, West Azerbaijan and East Azerbaijan provinces located in northwest of Iran. Peripheral blood samples were aseptically taken from the jugular vein and transferred into venojects containing sodium heparin. The method of lymphocyte culturing was used for the current study. The present procedure was a combination of Moorheah *et al.* (1960) and Lin *et al.* (1970).

Lymphocyte culture: For this purpose, 4.5 ml of RMPI 1640 medium was prepared with 2% phytohemagglutinin (PHA) as a mitogen and transferred in the culture tube. Then 0.5 ml of blood sample per animal was dropped into a tube, incubated at 37ºC under 5% of CO_2 environment and regularly shaken.

Cell harvesting and staining: At the 70th hour of incubation, Colcemid was added as mitotic inhibitor and tubes incubated at 37°C for 2 hours .Tubes were centrifuged at 1100 RPM for 20 minutes. Supernatant was removed and the pellet (containing white and red blood cells) was kept. Then, 0.075 M Potassium chloride as standard hypotonic solution was added to the pellet after exposure to colcemid. The cells incubated for 35 minutes. Potassium chloride was discarded, cells were fixed by cool fixative (3methanol: 1 glacial acetic acid). Fixative lysed any red blood cells present in the samples and fixed the white blood cells (especially lymphocyte). Then the fixative was discarded. In this step, just white blood cells remained in the tubes. Beside, fixed cells (white blood cells) from

suspensions cultures were dropped onto clean glass slides by micropipette and well. After 5 days, the slides soaked into trypsin solution (35 mg trypsin in 70 ml PBS) at 37ºC for 30 seconds and washed into PBS solution for 30 seconds and the slides well dried. The slides stained with 20% Giemsa's solution for 25 minutes (Gersen *et al., 2005*).

Chromosomal analysis: Chromosome counting was performed on metaphase cells under the light microscope. Fifteen clearly observable spread of each sample picked out and then photographed. The length of the short arm (Ls), length of the long arm (Ll), length of each chromosome (LT) and centromeric index (CI) were measured using MicroMeasure 3.3 (this software is made by the Deartment of Colorado, USA). Other parameters including relative length (RL) were calculated by Microsoft Excel 2013.The relative length is expressed by percentage andis the ratio obtained by dividing the whole length of any individual chromosome to the total length of all the chromosomes in the haploid set. The centromeric index was computed to categorize the types of chromosomes according to Guerra (1986). The Karyograms and the idiograms were drawn using Adobe Photoshop CS6 and Microsoft excel 2013, respectively.

Results

The results of this study show that there are no difference in chromosome number and type between Mazani and Azeri buffaloes (Figures 1 and 2).

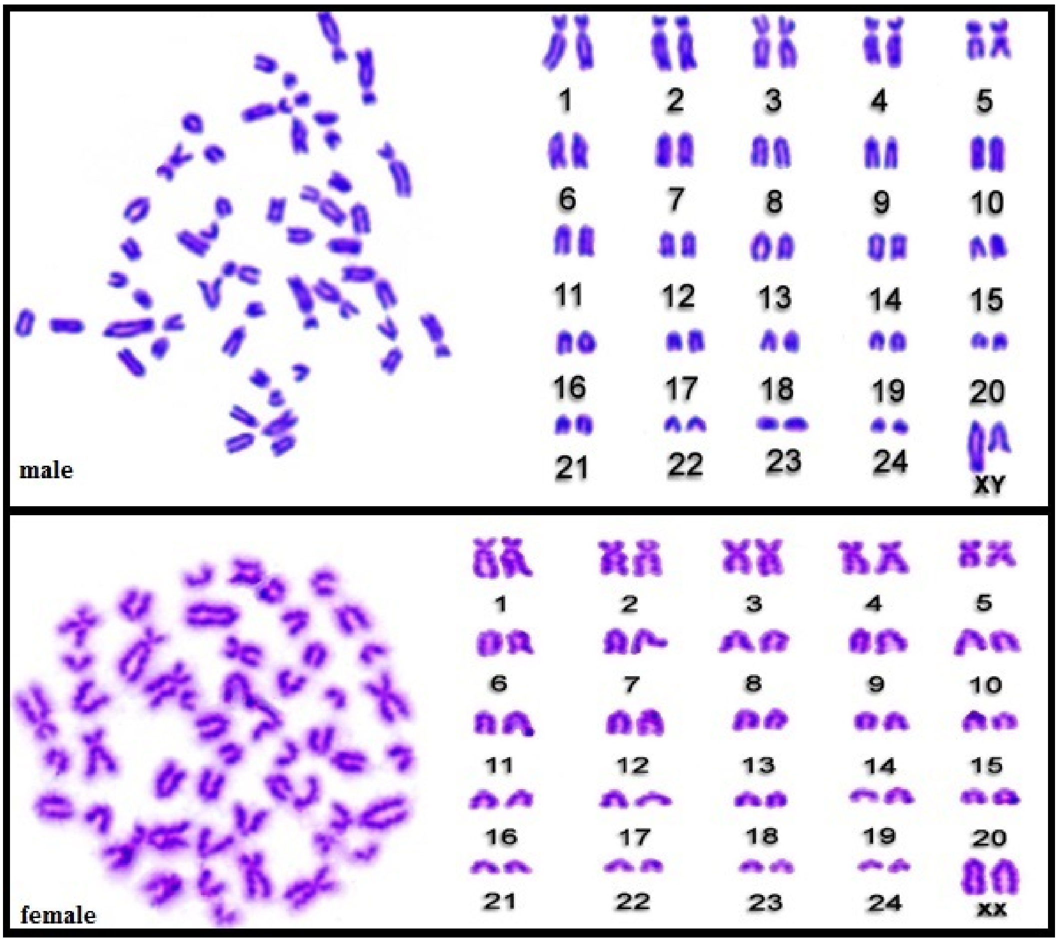


Figure 1. Chromosome spread (left) and karyotype (right) of male (up) and female (down) Mazani buffalo (*Bubalusbubalis*) 2n (diploid) = 50, by conventional staining method (×1000).

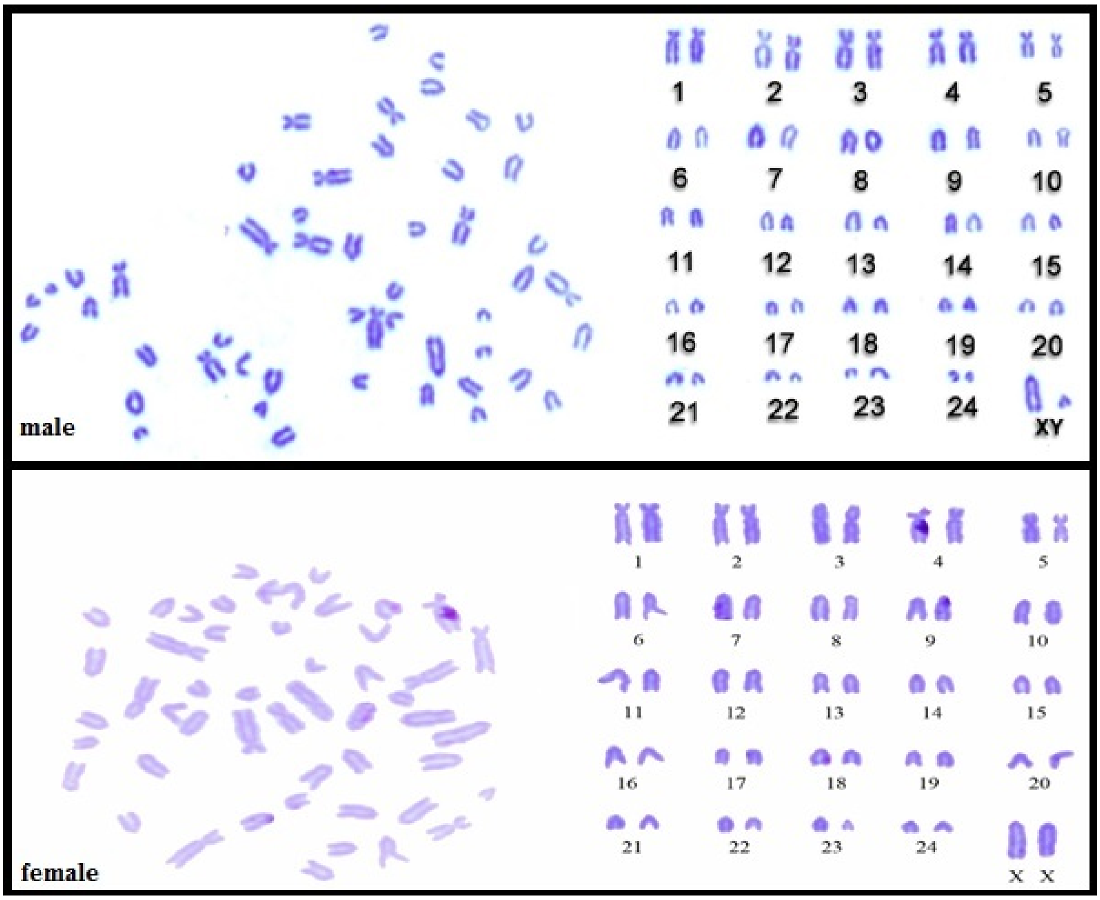


Figure 2. Chromosome spread (left) and karyotype (right) of male (up) and female (down) Azeri buffalo (*Bubalusbubalis*) 2n (diploid) = 50, by conventional staining method (×1000).

Diploid chromosomes (2n) of both Mazani and Azeri buffalo are 50 and fundamental number (NF) of Mazani and Azeri buffalo is 60 in male and female. So,based on these observations, both of them are riverine (*Bubalusbubalisbubalis*). The autosomes consist of 6 submetacentric, 4 metacentric and 38 telocentric chromosomes (pair Nos. 6-24) in Mazani and Azeri buffalo. The pair of sex chromosomes was XX in the female and XY in the male. From both karyotypes it appeared that the X was the largest telocentric while the Y was one of the smallest telocentric.

The mean of the short arm (Ls), long arm (Ll), chromosome length (LT), relative length (RL), arm ratio (Ll/Ls) and centromeric index (CI) of Mazani and Azeri buffalo are shown in Table 1 and 2, respectively.

Table 1. Mean of the short arm (Ls), long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) from metaphase chromosomes of Mazani male and female buffalo.

Chromosome pairs	Ls(μm)	Ll(μm)	LT(μm)	CI	RL*	Type of Chromosome
1	5.46	11.53	16.99	0.32	7.20	Submetacentric
2	4.34	11.36	15.70	0.28	6.66	Submetacentric
3	5.17	9.16	14.33	0.36	6.08	Submetacentric
4	6.02	8.07	14.09	0.43	5.97	Metacentric
5	5.16	7.49	12.65	0.41	5.36	Metacentric
6	0.00	9.80	9.80	0.00	4.16	Telocentric
7	0.00	9.56	9.56	0.00	4.05	Telocentric
8	0.00	9.26	9.26	0.00	3.93	Telocentric
9	0.00	8.93	8.93	0.00	3.79	Telocentric
10	0.00	8.74	8.74	0.00	3.71	Telocentric
11	0.00	8.46	8.46	0.00	3.59	Telocentric
12	0.00	8.38	8.38	0.00	3.55	Telocentric
13	0.00	7.88	7.88	0.00	3.34	Telocentric
14	0.00	7.34	7.34	0.00	3.11	Telocentric
15	0.00	7.32	7.32	0.00	3.10	Telocentric
16	0.00	7.26	7.26	0.00	3.08	Telocentric
17	0.00	7.03	7.03	0.00	2.98	Telocentric
18	0.00	6.90	6.90	0.00	2.93	Telocentric
19	0.00	6.40	6.40	0.00	2.71	Telocentric
20	0.00	6.18	6.18	0.00	2.62	Telocentric
21	0.00	5.75	5.75	0.00	2.44	Telocentric
22	0.00	5.54	5.54	0.00	2.35	Telocentric
23	0.00	5.31	5.31	0.00	2.25	Telocentric
24	0.00	5.11	5.11	0.00	2.17	Telocentric
X	0.00	14.52	14.52	0.00	6.16	Telocentric
Y	0.00	6.40	6.40	0.00	2.71	Telocentric

*Relative length is expressed by percentage andis the ratio obtained by dividing the whole length of any individual chromosome to the total length of all the chromosomes in the haploid set.

Table 2. Mean of the short arm (Ls), long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) from metaphase chromosomes of Azeri male and female buffalo.

Chromosome pairs	Ls(μm)	Ll(μm)	LT(μm)	CI	RL	Type of Chromosome
1	9.29	12.46	21.75	0.43	6.55	Metacentric
2	7.65	13.54	21.19	0.36	6.38	Submetacentric
3	5.82	13.56	19.38	0.30	5.83	Submetacentric
4	6.45	11.43	17.88	0.36	5.38	Submetacentric
5	7.63	9.04	16.67	0.46	5.02	Metacentric
6	0.00	14.71	14.71	0.00	4.43	Telocentric
7	0.00	14.49	14.49	0.00	4.36	Telocentric
8	0.00	13.05	13.05	0.00	3.93	Telocentric
9	0.00	12.95	12.95	0.00	3.90	Telocentric
10	0.00	12.87	12.87	0.00	3.87	Telocentric
11	0.00	12.62	12.62	0.00	3.80	Telocentric
12	0.00	12.33	12.33	0.00	3.71	Telocentric
13	0.00	12.05	12.05	0.00	3.63	Telocentric
14	0.00	11.64	11.64	0.00	3.50	Telocentric
15	0.00	11.24	11.24	0.00	3.38	Telocentric
16	0.00	10.91	10.91	0.00	3.28	Telocentric
17	0.00	10.17	10.17	0.00	3.06	Telocentric
18	0.00	10.08	10.08	0.00	3.03	Telocentric
19	0.00	9.85	9.85	0.00	2.97	Telocentric
20	0.00	8.48	8.48	0.00	2.55	Telocentric
21	0.00	8.11	8.11	0.00	2.44	Telocentric
22	0.00	7.7	7.70	0.00	2.32	Telocentric
23	0.00	7.51	7.51	0.00	2.26	Telocentric
24	0.00	7.34	7.34	0.00	2.21	Telocentric
X	0.00	18.34	18.34	0.00	5.52	Telocentric
Y	0.00	8.86	8.86	0.00	2.67	Telocentric

The relative length of chromosomes ranged between 1.32 and 6.72 in Mazani buffalo (Table 1), and ranged between 1.81 and 7.19 in Azeri buffalo (Table 2). It means difference of range relative length (DRL) is 5.4 in Mazani buffalo and 5.38 Azeri buffalo. Also, the chromosome length ranged Between 8.75 to 44.57 in Mazani buffalo, and ranged Between 9.20 to 36.52 in Azeri buffalo.

The idiogram of Mazani and Azeri buffalo are presented in figure 3 and these show there are minimal differences between Mazani and Azeri buffalo idiograms.

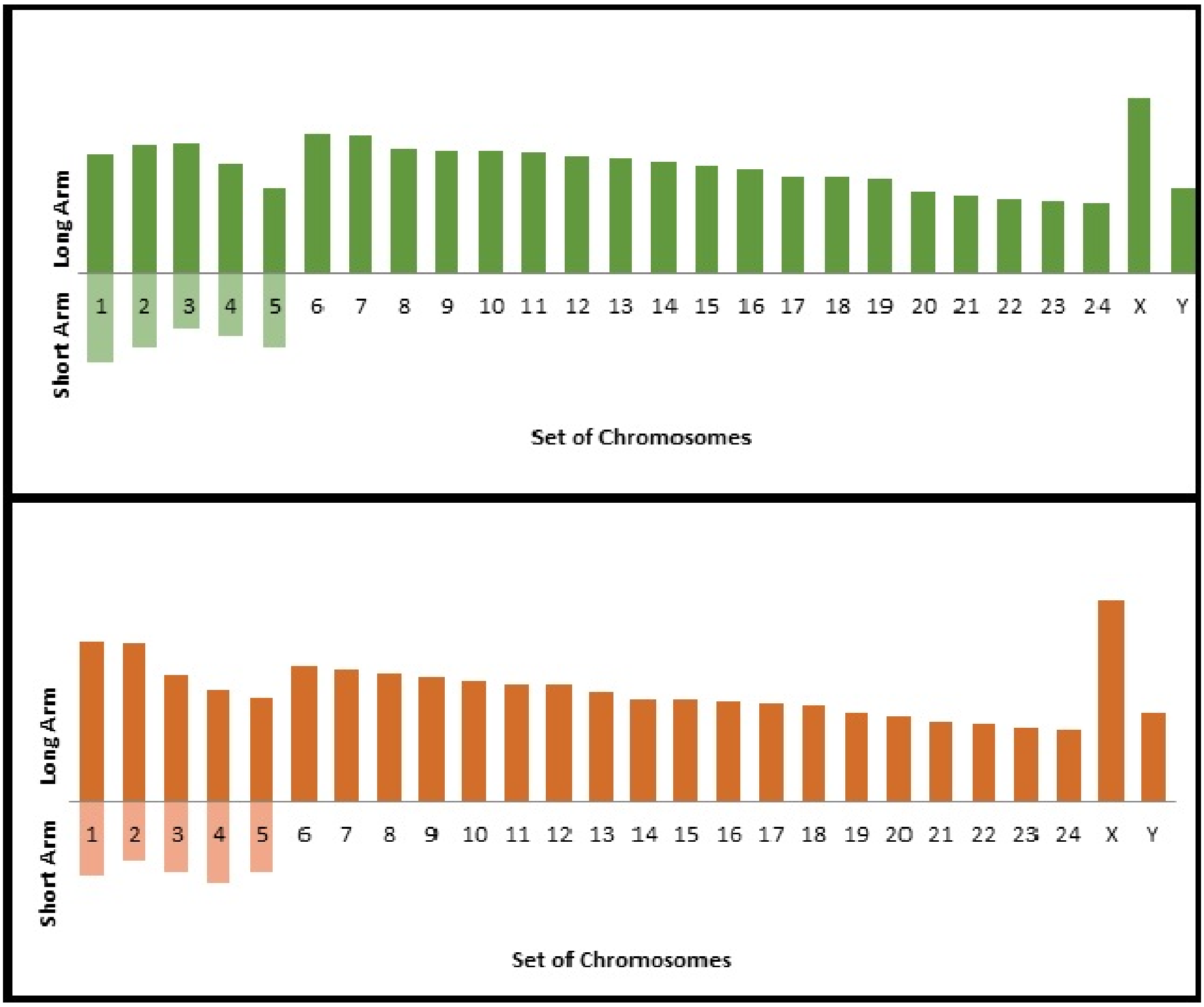


Figure 3.The idiogram of Mazani (up) and Azeri (down) buffalo.

All chromosomes from both populations were found normal. It means we did not see any chromosome abnormalities like trisomy and monosomy in karyotypes. The karyotype formula of Mazani and Azeri buffaloes is as follows:

2n (50) = 6 submetacentric + 4 metacentric + 38 Telocentric + Sex chromosomes

Discussion

The current study showed that diploid chromosomes (2n) of both Mazani and Azeri buffalo were 2n=50. So, based on these observation both of them must be riverine (*Bubalus bubalis bubalis*). The results of this study confirmed the findings of previous

works in this field. In fact, the findings of the current study are consistent with those of Khavari (1978) who found that the diploid number of river buffalo in Iran is 50. There are similarities between diploid number in this study and the other studies on river buffalo in Brazil (Rommelt, 1976; Pires *et al.*, 1997), India (Gupta and chaudhri, 1978; Yadav *et al.*, 1984; Balakrishnan and Yadav, 1984; Bidhar *et al.*, 1986; Kumar and Yadav, 1991; Ramesha and Hedge 1992; Joshi and Govindaiah, 1999; Murali *et al.*, 2009), Pakistan (Ali *et al.*, 2012), Thailand (Kenthao *et al.*, 2012) and Egypt (De Hondt and Ghanam, 1971; Cribiu and Obeidah, 1978; Ahmed *et al.*, 2004). It's similar to Halnan (1976) that reported the diploid number is 50. The current result is not similar to the previous studies that showed the diploid number is 2n = 48 in swamp buffalo in Japan (Miyake *et al.*, 1980; Harisah *et al.*, 1989), Australia (Toll and Halnan, 1976a), Malaysia (Bongso and Jainudeen, 1979) and Thailand (Kenthao *et al.*, 2012).

The fundamental number (NF) of Mazani and Azeri buffalo was found to be 60 in male and female and it is in agreement with De Hondt and Ghanam (1971) Bongoso *et al.* (1977); Iannuzzi (1994) and Kenthao *et al.* (2012) that found the NF is 60 in river buffalo.

The results of this study indicate that the autosomes consist of 10 submetacentric / metacentric (pair Nos. 1-5) and 38 telocentric chromosomes (pair Nos. 6-24) in Mazani and Azeri buffalo. This is similar to Kenthao *et al.* (2012) who reported that there are 10 submetacentric and 38 telocentric in Mehsani buufaloes. However; this is different from the study that reported the autosomes of Pakistani river buffalo contain 10 metacentric / submetacentric chromosomes whereas the rest of the autosomes were classified as acrocentric ones (Ali *et al.*, 2012). Cribiu (1987) reported the autosomes of Egyptian river buffalo contain 5 pairs metacentric / submetacentric chromosomes and 19 pairs acrocentric chromosmes.

The pair of sex chromosomes was XX in the female and XY in the male. From both karyotypes it is apparent that the X was the largest telocentric, while the Y was one of the smallest telocentric chromosomes. In the current study we recognized the type of chromosomes using MicroMeasure 3.3. This finding is in agreement with Kenthao*et al.* (2012) that reported the X is largest telocentric and Y is one of the smallest telocentric in Mehsani buffaloes from Thailand. They used of $AgNo_3$ Banding and this method is very useful for distinguish difference between acrocentric and telocentric chromosomes (Gersen *et al.*, *2005*). The previous reports showed the X chromosome is the largest acrocentric and Y chromosome is one of the smallest ones in Pakistaniriver buffalo (Ali *et al.*, 2012), Indian river buffalo (Murali *et al.*, 2009; Nair *et al.*, 1986; Iannuzzi, 1994), Brazilian river buffalo (Pires *et al.*, 1997) and Egyptian river buffalo (Cribiu, 1978). The current study is different from Meo *et al.*, (2005) that reported the Y chromosome is acrocentric in river buffalo. It is because they guess the type of chromosomes just from pictures and did not use from any kind of techniques and software's.

Our results demonstrates that the relative length (RL) of chromosomes ranged between and 2.17 to 7.20 in Mazani buffalo, and ranged between 2.21 to 6.55 in Azeri buffalo (Table 1 and 2). The means difference relative length (DRL) is 5.03 in Mazani buffalo and 4.34 Azeri buffalo. These results were very different from DRL of Toda buffalo that is 3.95 (Murrali *et al.*, 2009). Also, the results show that the chromosome length ranged between 16.99 and 5.11 in Mazani buffalo, and ranged between 21.75 and 7.34 in Azeri buffalo.

It is concluded that, both of Mazani and Azeri buffaloes are riverine and there is no differences between karyotype of Mazani and Azeri buffaloes.

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آنالیز کروموزومی دو نژاد ازگاومیش های مازنی وآذری ایران

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چکیده

در این پژوهش، کاریوتایپ جمعیت گاومیش های مازنی در مقایسه با جمعیت گاومیش های آذری مورد مطالعه قرار گرفت. نمونه هـای خونی از ده (۵ نر و ۵ ماده) گاومیش مازنی و سی (۱۵نر و ۱۵ ماده) گاومیش آذری جمع آوری شد. گاومیش های مازنی متعلق بـه اسـتان مازندران و گاومیش های آذری متعلق به استان های آذربایجان غربی و شرقی و اردبیل هستند. نمونه های خـونی در دمـای ۳۷ درجـه بـه مدت ۷۲ ساعت و در حضور فیتوهماگلوتینین کشت داده شدند و گستره های متافازی بر روی لام تهیه گردید. از گیمسا برای رنـگ آمیـزی کروموزوم ها استفاده شد. گاومیش مازنی و آذری کاریوتایپ مشابه با عدد دیپلوئید $2n = 50$ را نشان دادند. تعداد بازوان (NF) در جـنس نر و ماده، ۶۰ بدست آمد. انواع کروموزوم شامل ۶ ساب متاسانتریک، ۴ متاسانتریک و ۴۰ تلوسانتریک بوده کـه کرومـوزوم X بزرگتـرین کروموزوم تلوسانتریک و کروموزوم Y یکی از کوچکترین کروموزوم های تلوسانتریک بدست آمد. طول نسـبی کرومـوزوم هـا در گـاومیش مازنی، بین ۲/۱۷ تا ۷/۲۰ و در گاومیش آذری بین ۲/۲۱ تا ۶/۵۵ متغیر بود. همه کروموزوم های هر دو جمعیت طبیعی بودنـد. بنـابراین، بـر اساس کاریوتایپ تعیین شده،گاومیش های مازنی و آذری هر دو رودخانه ای هستند .

واژگان کلیدی:کاریوتیپ، کروموزوم، گاومیش رودخانه ای، ایدیوگرام